Parasitism of *Pratylenchus* spp. to 'Lovell,' 'Nemaguard' and 'Okinawa' Peach

By

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Abstract of Dissertation Presented to the
Graduate Council of the University of Florida in Partial Fulfillment
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PARASITISM OF PRATYLENCHUS SPP. TO
'LOVELL,' 'NEMAGUARD' AND 'OKINAWA' PEACH

By

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Three species of Pratylenchus were demonstrated to parasitize and reproduce in roots of 'Lovell,' 'Nemaguard' and 'Okinawa' peaches, Prunus persica Batsch. Populations of P. brachyurus (Godfrey, 1929) Filipjev and Schuurmans Stekhoven, 1941, P. coffeae (Zimmerman, 1898) Goodey, 1951, and P. penetrans (Cobb, 1917) Chitwood and Oteifa, 1952, increased when inoculated onto these peaches.

Two tests for pathogenicity of P. brachyurus were conducted using inoculum levels of 100 and 1,000 specimens per plant. 'Okinawa,' 'Lovell' and 'Nemaguard' peach were included in the low inoculum test, but 'Nemaguard' was not tested at the high inoculum level. Substantial nematode population increases resulted on each peach variety of each test. Statistical comparisons of plant dry weights (top, root, and total) disclosed insignificant differences between inoculated and uninoculated plants of all the rootstock varieties used in the 100 (low) inoculum level test. In the 1,000 (high) inoculum level

test, however, statistical significance at the 1 per cent level occurred between dry weights of tops, roots, and total plants of 'Okinawa.' Statistical significance was also obtained at the 5 per cent level on root dry weights of 'Lovell' in this test.

Roots of inoculated plants in three tests exhibited necrotic lesions. No differences in types of lesions were noted among the various peach rootstocks.

Microscopic examination of sections of each peach rootstock revealed damage to cortical parenchyma by P. brachyurus. The tissue damage varied from a slight effect on several cells to the formation of cavities due to destruction of many cells. All developmental stages of P. brachyurus were found associated with various degrees of tissue damage.

Results of the tests indicated that large numbers of Pratylenchus brachyurus may have little effect on the growth of 'Lovell' and 'Okinawa' peaches.

A nematophagous fungus, identified as Arthrobotrys anchonia Drechsler, was found associated with Pratylenchus brachyurus in greenhouse-maintained colonies.

Pratylenchus brachyurus males reared on peach did not have a small rib formed by phasmids extending into the bursa. This conforms to the original description but not to the description of P. brachyurus males recovered from citrus in Florida.

Projections extending anteriorly from the stylet knobs were observed in some populations of P. brachyurus when reared on 'Dixie 18'

field corn but not on 'Lovell' peach or sweet orange, Citrus sinensis Osbeck. Progeny of the nematodes with this characteristic did not possess the projections when reared on peaches or citrus.

INTRODUCTION

Several factors have until recently prevented the development of a major peach-growing industry in Florida. Peach, Prunus persica Batsch, is susceptible to the root-knot nematode species Meloidogyne incognita (Kofoid and White, 1919) Chitwood, 1949 and M. javanica (Treub, 1885) Chitwood, 1949, the dominant root-knot nematodes in Central and North Florida. 'Lovell' peach, highly susceptible to both root-knot nematode species, was a common rootstock in these areas (26). Resistance in peach rootstocks to both of these species was made available with the introduction of the 'Okinawa' and 'Nemaguard' rootstock varieties. Scion varieties used for fruit production normally are propagated onto rootstocks by bud grafts. These rootstocks, plus other research developments, have recently made possible economic production of peaches in Florida. Malo (20) found that the nature of resistance of 'Nemaguard' and 'Okinawa' peach rootstocks to M. javanica lies in the ability of the host plant to inhibit nematode growth. This is accomplished by walling off the nematodes and suppressing development of giant cells (or nurse cells) on which the nematode feeds. Little or no attention has been devoted to the reaction of Pratylenchus spp. on the root-knot nematode resistant 'Okinawa' and 'Nemaguard' peach rootstocks.

The nematode genus Pratylenchus Filipjev contains certain obligately plant parasitic species which rank among the more important organisms responsible for plant root destruction (29).

Steiner (28) ranked *Pratylenchus* spp. as tissue parasites "par excellence" and major economic pests. A recent key (5) lists 35 species of *Pratylenchus*.

Principal factors influencing distribution of *Pratylenchus* species are climate and soil type (17). *Pratylenchus* spp. were recovered from over 95 per cent of 215 samples of varied host and representative soil types collected from Gainesville to Homestead, Florida (8).

Steiner (28) stated that under certain conditions *Pratylenchus* may multiply to enormous numbers, particularly in orchards that remain undisturbed for years, but also on some annual crops such as peanuts.

Species of *Pratylenchus* found in Florida associated with economic crop plants include *P. brachyurus* (Godfrey, 1929) Filipjev and Schuurmans Stekhoven, 1941, *P. coffeae* (Zimmerman, 1898) Goodey 1951, and *P. penetrans* (Cobb, 1917) Chitwood and Oteifa, 1952. *P. brachyurus* is the most commonly observed species of the genus in Florida (8) and is often recovered from soil and root samples taken from peach trees.[1] *P. penetrans* has been identified as an agent in the peach replant problem in Canada (22) and causes appreciable injury to roses grafted on *Rosa fortuneana* Lindley stock in Florida (19). *P. coffeae* stunted young apple trees and caused root injury to peach trees and other fruit crops when planted in infested soil (3). This nematode is associated with several economic plants in Florida.[1]

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[1]Unpublished records of Nematology Bureau, Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville.

Since *P. brachyurus*, *P. coffeae*, and *P. penetrans* are prominent parasitic nematodes, the possibility exists that they may cause damage to peach roots. Since 'Okinawa' and 'Nemaguard' rootstocks show resistance to *M. incognita* and *M. javanica*, it is important to determine the reaction of other plant parasitic nematodes to these rootstocks.

The primary objective of this study was to determine if *P. brachyurus*, *P. coffeae*, and *P. penetrans* parasitized, reproduced upon, and caused damage to 'Lovell,' 'Okinawa' and 'Nemaguard' peach rootstocks. Secondary objectives were to study the general morphology, life cycle stages, and feeding habits of *P. brachyurus*.

LITERATURE REVIEW

Since 1880 when the first species of *Pratylenchus* was described as *Tylenchus pratensis* by de Man (21) a total of 34 species has been described. Filipjev (9) erected *Pratylenchus* in 1936. A monograph of the genus was published by Sher and Allen (27) in 1953. They listed the following synonyms for *P. coffeae*:

Tylenchus coffeae Zimmerman, 1898

Tylenchus musicola Cobb, 1919

Pratylenchus musicola (Cobb, 1919) Filipjev, 1936

Tylenchus mahogani Cobb, 1920

Anguillulina mahogani (Cobb, 1920) Goodey, 1932

Pratylenchus mahogani (Cobb, 1920) Filipjev, 1936

Anguillulina pratensis Goffart, 1929

P. coffeae attacks a wide range of economic plants including camellia, vetch, and strawberry in the United States (29). Table 2 gives a partial list of host plants of *P. coffeae* in Florida. Colbran (4) reported it to be associated with *Prunus persica* Batsch in Australia.

Pratylenchus penetrans (Cobb, 1917) Chitwood and Oteifa, 1952, had been named *Tylenchus penetrans* earlier by Cobb (17).

Records indicate that *P. penetrans* has one of the widest known host ranges of any species in the genus (29). In Canada, *P. penetrans* has been defined as a serious problem in peach replant (22).

Table 1. A host list of Pratylenchus brachyurus in Florida compiled from the unpublished files of the Nematology Bureau, Florida Department of Agriculture and Consumer Services, Gainesville, Florida

Scientific Name	Common Name
Acer saccharinum L.	white maple
Aleurites Fordii Hemsl.	tung oil tree
Aloe sp.	
Annona glabra L.	pond apple
Arecastrum Romanzoffianum Becc.	green palm
Asclepias tuberosa L.	butterfly weed
Asimina parviflora (Michx.) Dural.	papaw
Begonia sp.	
Carya illinoensis Koch	pecan
Cassia sp.	
Chamaecrista sp. Moench.	
Chamaecyparis obtusa Sieb. & Zucc.	Hinoki cypress
Chamaedorea elegans Mart.	parlor palm
Chrysanthemum sp.	
Citrus sp.	
Citrus aurantium L.	sour orange
Citrus limon Burm.	lemon
Citrus paradisi Macf.	grapefruit
Citrus reticulata Blanco	tangerine

Table 1. (continued)

Scientific Name	Common Name
Citrus sinensis Osbeck	sweet orange
Cocos nucifera L.	coconut palm
Codiaeum sp. Juss.	
Cynodon dactylon Pers.	Bermuda grass
Digitaria sanguinalis (L.) Scop.	crabgrass
Diospyros virginiana L.	common persimmon
Eremochloa ophiuroides Hack.	centipede grass
Eupatorium capillifolium (Lam.) Small.	dog fennel
Euphorbia sp.	
Euphorbia pulcherrima Willd.	poinsettia
Fragaria sp.	
Gladiolus sp.	
Glottidium vesicarium (Jacq.) Harper	coffee weed
Hemerocallis fulva L.	common orange daylily
Hibiscus sp.	
Hibiscus rosa-sinensis L.	Chinese hibiscus
Indigofera endecaphylla Jacq.	indigo
Juglans nigra L.	black walnut
Jussiaea peruviana L.	primrose willow
Lantana sp.	

Table 1. (continued)

Scientific Name	Common Name
Ligustrum sp.	
Ligustrum lucidum Ait.	glossy privet
Lonicera sp.	
Lycopersicon esculentum Mill.	tomato
Magnolia grandiflora L.	bull bay
Meibomia sp.	
Menthe spicata L.	spearmint
Myrica cerifera L.	wax myrtle
Paspalum notatum Flugge.	Bahia grass
Paspalum urvillei Steud.	
Pennisetum glaucum R. Br.	pearl millet
Persea americana Mill.	avocado
Philodendron sodiroi Hort.	philodendron
Phoenix Roebelenii O'Brien	dwarf date-palm
Phytolacca americana L.	pokeberry
Pinus clausa (Engelm.) Vassey.	sand pine
Pinus elliottii Engelm.	slash pine
Pinus palustris Mill.	longleaf pine
Polystichum adiantiforme J. Smith	leather leaf fern

Table 1. (continued)

Scientific Name	Common Name
Prunus persica Batsch	peach
Psidium guajava L.	guava
Pteridium aquilinum (L.) Kuhn	
Pteris latiuscula Desv.	
Pyrus sp.	
Quercus laevis Walt.	turkey oak
Saccharum officinarum L.	sugar cane
Saintpaulia ionantha Wendl.	African violet
Schinus terebinthifolius Redd.	Brazilian pepper tree
Serenoa repens (Barth.)	saw-palmetto
Smilax sp.	
Stenotaphrum secundatum Kuntze	St. Augustine grass
Stillingia angustifolia (Torr.) S.Wats.	queen's-root
Vigna sinensis 'Iron' (L.) Endl.	cowpea
Zoisia japonica Steud.	Japanese lawn grass
Zoisia japonica 'Emerald' Steud.	

Table 2. A host list of *Pratylenchus coffeae* in Florida compiled from the unpublished files of the Nematology Bureau, Florida Department of Agriculture and Consumer Services, Gainesville, Florida

Scientific Name	Common Name
Aglaonema simplex Blume	
Ananas comosus Merr.	pineapple
Chamaedorea seifrizii Burret	parlor palm
Citrus sp.	
Hedera canariensis Willd.	Algerian ivy
Hibiscus sp.	
Ilex glabra Gray	gallberry
Monstera deliciosa Liebm. (Ceriman)	
Pinus elliottii Engelm	slash pine
Quercus laurifolia Michx.	laurel oak
Trifolium hybridum L.	alsike clover
Vriesia imperialis Carriere	bromeliad

Table 3. A host list of Pratylenchus penetrans in Florida compiled from the unpublished files of the Nematology Bureau, Florida Department of Agriculture and Consumer Services, Gainesville, Florida

Scientific Name	Common Name
Aglaonema commutatum Schott.	
Chrysanthemum sp.	
Eremochloa ophiuroides Hack.	centipede grass
Ficus pandurata Sander	fiddleleaf fig
Ilex glabra Gray	gallberry
Juniperus silicicola (Small) Bailey	Southern red cedar
Pinus sp.	
Polystichum adiantiforme J. Smith	leather leaf fern
Zoisia sp.	Japanese lawn grass

In Florida, P. penetrans is parasitic to a rose rootstock, Rosa fortuneana Lindley (19), and leather leaf fern, Polystichum adiantiforme J. Smith (24). Other plants attacked by P. penetrans in Florida are listed in Table 3.

According to Loof (17), synonyms for P. brachyurus include:

Tylenchus brachyurus Godfrey, 1929

Pratylenchus leiocephalus Steiner, 1949

Pratylenchus pratensis Thorne, 1940

Pratylenchus steineri Lordello, Zamith and Boock, 1954

P. brachyurus is reported to attack many economic plants including pineapple, peanut, strawberry, lespedeza, cotton, okra, corn, tobacco, and potato tubers (29). Pratylenchus spp. have been the most frequently found plant parasitic nematodes associated with citrus roots (2). P. brachyurus is the species of the genus most commonly found in Florida (8) and it is reported pathogenic to citrus (1). Additional hosts of P. brachyurus in Florida are listed in Table 1.

Fliegel (10) reported Pratylenchus vulnus, P. zeae and P. brachyurus on peach in Georgia. P. brachyurus, the most commonly found species, was not associated with severe damage to roots and populations were relatively small. P. zeae generally did not affect plant root systems.

Godfrey (11) characterized symptoms of pineapple roots caused by P. brachyurus as irregularly elongate brown spots which are light in color when young and later become darker. Lesions are not sunken and are readily distinguishable from lesions produced by other organisms. Discoloration and cell destruction were observed from the epidermal cells through the cortex.

Brooks and Perry (1) reported that injury by P. brachyurus extended two to three cells to each side of the nematode, which suggests toxic substances secreted by the nematode.

Graham (12) reported damage to tobacco and corn by Pratylenchus brachyurus and P. zeae. These two nematode species were always found in the outer parenchyma and never in the vascular tissues.

In Pretoria, Koen and Hogewind (16) observed that Pratylenchus brachyurus caused irregularly shaped purple-brown lesions 1-5 mm in diameter on potato tubers. Infected tubers lose considerable weight when stored at room temperatures and are unsuitable for use as seed pieces. By digging potatoes at an early stage and storing at 5 C no visible lesions developed.

Pratylenchus vulnus was reported (18) to be associated with declining peach orchards in California. D'Souza (6) reported P. vulnus as a pathogen of peach in California; however, the nematode appeared most prominent as a disease incitant or a disease aggravator and alone did not stunt peach seedlings at the population levels occurring in peach orchards.

Feeding by Pratylenchus spp. is of two obvious types. One type (15) is exhibited by P. crenatus which feeds ectoparasitically on epidermal cells, migrating from cell to cell and causing light necrotic spots on the roots. Another type (23) is exhibited by P. scribneri, which feeds endoparasitically on amaryllis, forming large necrotic areas in the cortex with "nests" of eggs and larvae.

MATERIALS AND METHODS

Soil and Inoculum Sources

Soil for all experiments was obtained from the "Agronomy Farm," Institute of Food and Agricultural Sciences, University of Florida, Gainesville, and was of the Lakeland fine sand series. The soil used in each experiment was fumigated with methyl bromide (2 pounds per cubic yard) and aerated at least 3 weeks before use.

The three species of *Pratylenchus* under investigation were obtained from different sources. Specimens of *Pratylenchus brachyurus* were obtained from peach growing in a nursery near Belleview, Florida; *P. penetrans* from leather leaf fern, Crescent City, Florida; and *P. coffeae* from Chinese evergreen growing in a greenhouse at Gainesville, Florida.

General Methods

Populations of *P. brachyurus* used as inoculum were maintained and increased on 'Lovell' peach; 'Dixie 18' field corn, *Zea mays* L.; or sweet orange, *Citrus sinensis* Osbeck. *P. penetrans* and *P. coffeae* populations were maintained and increased on the hosts from which they had been collected, leather leaf fern and Chinese evergreen, respectively. Inoculated host plants used for colonizing respective nematode species were grown in wooden boxes, approximately 12 x 24 x 10 inches in dimension, containing fumigated soil. Adequate quantities of nematodes for

inoculum were thus always available when needed. All colonization and pathogenicity tests were conducted in an environmental chamber at 24 ± 2 C and illuminated 2200 ft c for 16 hours per day.

Nematode specimens used for inoculum were obtained by incubating appropriate infected plant roots for about 2 days in glass pint jars containing a small amount of water. Root washings from the jars were placed onto a facial tissue in a Baermann funnel partially filled with distilled water and the nematodes were recovered in the bottom of the funnel. Nematodes recovered were subsequently hand-picked into distilled water before use. A ratio of nine females to one male was used for P. penetrans and P. coffeae where males are plentiful, and only females, of course, for P. brachyurus where males are rare.

Identification of Nematode Species

Using the following method Pratylenchus spp. were identified both at the beginning of the test and after plants were harvested.

Specimens were hand-picked into distilled water on a glass slide. The slide was moved about over a small gas flame for 5-6 seconds duration and repeated until the nematodes ceased twisting and straightened out. Glass rods of a diameter near that of the nematodes were then placed in a triangle around the specimens and a cover glass applied. Zut was used to seal the edges of the cover glasses to the slides. Other specimens so killed were fixed in 3 per cent formalin and mounted in formalin. Some of those fixed in the 3 per cent formalin were processed to anhydrous glycerine and mounted, using

Seinhorst's method (25). Specimens from each method were then studied in detail with the aid of a research microscope equipped with an oil immersion objective.

Experiment I

Peach seedlings of 'Lovell,' 'Okinawa,' and 'Nemaguard' varieties grown in vermiculite (Fig. 1) to a height of about 2 cm were obtained from the Fruit Crops Department, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, for experimental purposes. When seedlings were approximately 3 cm in height each was transplanted into a 6-inch clay pot partially filled with fumigated soil. Each peach variety was replicated three times for each of the three nematode species. One pot of each peach variety served as an uninoculated control for each replicate.

The plants were inoculated with nematodes 16 days after transplanting, when they were approximately 4 cm in height. Fifty handpicked specimens of the appropriate *Pratylenchus* species in a water suspension were withdrawn into a 10 cc capacity hypodermic syringe (without needle) and ejected about three quarters of an inch deep into the soil of a designated pot. A thin layer of moistened sterilized soil was used to cover the existing soil and distilled water was added. This procedure was followed in all inoculations. Distilled water was added to each pot as needed to maintain adequate soil moisture. A liquid fertilizer solution (Ortho^R 12-6-6) at the rate of 1 tablespoon per gallon of water was applied every 2 weeks. Plants were sprayed with Kelthane as needed to control mites. The plants were visually inspected every day.

Fig. 1. Photograph of young 'Nemaguard' seedlings growing in vermiculite.

Sixteen weeks after inoculation, the plant tops were removed just above the soil line and discarded. Roots were removed from the soil and two 5-gram samples (net weight) were placed into a food blender and comminuted for approximately 20 seconds. The blender contents were then washed onto a facial tissue held in a Baermann funnel partially filled with distilled water and left overnight. The nematode suspension was drawn from the Baermann funnel and poured onto a 325-mesh sieve. The sievings were washed into a Syracuse watch glass and the nematodes counted with the aid of a dissecting microscope. Soil was mixed thoroughly and two-100 ml samples were processed by a centrifugal-flotation technique (13). Nematodes recovered were placed into a Syracuse watch glass and counted. The total number of nematodes per pot was calculated.

Experiment II

Five replicates of 'Lovell,' 'Okinawa,' and 'Nemaguard' peach approximately 4 cm high, growing in 8-inch clay pots partially filled with sterilized soil, were each inoculated with 100 hand-picked *Pratylenchus brachyurus*. This experiment was of 17 weeks duration.

Watering, fertilizing, spraying for pest control, and inspection for Experiments II and III were as described for Experiment I.

Seventeen weeks after inoculation tops were removed from plants. Roots were individually removed from the soil and washed to remove adhering soil particles. The washings were collected in a 2-liter capacity can, were roiled, and a 500 ml sample removed and processed by the centrifugation-flotation technique (13). Root washings in Experiment I were not processed separately, but included with soil processing.

The soil recovered from each treatment was mixed thoroughly, a 100-ml sample removed and processed by the centrifugation-flotation technique.

Nematodes recovered from root washings and soil were counted with the aid of a dissecting microscope and the total number of specimens per replicate was calculated.

Roots were incubated (30) by placing each replicate individually in a 4-pound ($4\frac{1}{2}$ x $3\frac{1}{2}$ x 13 inches) polyethylene bag. After 3, 7, 14, 21, and 28 days, emerged nematodes were collected by washing the roots and the interior of the polyethylene bags with tap water by a jet nozzle attachment. Washings were collected in cans and poured onto a 325-mesh screen. Nematodes collected on the screen were washed into Syracuse watch glasses for counting. Examinations and counting of nematodes were accomplished with the aid of a dissecting microscope. Total numbers of nematodes from each root system was obtained from roots, root washings, and soil samples.

Plant tops and roots (after incubation) were maintained for 2 weeks at 92 C in a plant-drying oven to obtain dry weights. Identity of tops and roots was maintained throughout the experiments.

Experiment III

Material and methods for Experiment III were the same as for Experiment II except 1,000 hand-picked *Pratylenchus brachyurus* per replicate were used and only 'Okinawa' and 'Lovell' peach were included.

All handling of plants and soil was the same as for Experiment II.

Histopathology of Peach Roots Infected With Pratylenchus brachyurus

Peach roots infected and not infected by P. brachurus were killed and fixed in FAA (formalin, 6.5 ml; glacial acetic acid, 7.5 ml; 50 per cent ethanol, 100 ml), dehydrated to tertiary-butyl alcohol and embedded in paraffin. Sections cut 12 µ thick with a rotary microtome were affixed to glass microscope slides with Haupt's adhesive, stained with safranin-fast green and mounted in Fisher's "Permount" according to Johansen's (14) procedure.

RESULTS AND DISCUSSION

Experiment I

Plant growth was virtually uniform throughout the 16-week experiment and no noticeable differences occurred on the above-ground plant parts. Each replicate in each treatment yielded greater nematode populations than had been used as inoculum, indicating parasitism and reproduction by *P. brachyurus*, *P. penetrans*, and *P. coffeae*. Using an average figure for all three rootstocks, *P. brachyurus* reproduced 3.2 times; *P. penetrans*, 2.7 times; and *P. coffeae*, 2.3 times the original inoculum levels (Table 4). And using an average figure for all three nematodes, 'Okinawa' peach supported the largest total populations, which was 3.3 times the inoculum level. 'Lovell' and 'Nemaguard' peach supported total nematode populations of 2.3 and 1.8 times, respectively, the inoculum level (Table 4).

P. brachyurus was chosen for further study because it reproduced at a greater rate than did *P. penetrans* or *P. coffeae*. *P. penetrans* and *P. coffeae* should not be discounted as potential pathogens of peach, because reproduction occurred and population increases were noted in 'Lovell,' 'Okinawa,' and 'Nemaguard' cultivars.

Roots of inoculated plants had very small brownish lesions that did not occur on roots of uninoculated plants.

Table 4. Numbers of *Pratylenchus brachyurus*, *P. penetrans*, and *P. coffeae* recovered from the roots of three peach cultivars used as rootstocks inoculated with 50 nematodes per 6-inch pot

Peach		*P. brachyurus*	*P. penetrans*	*P. coffeae*	Rootstock totals
'Lovell'		212	79	127	418
'Lovell'		88	154	80	322
'Lovell'		149	112	45	306
	Total	449	345	252	1046
	Average	150	115	84	315
'Okinawa'		280	218	0	498
'Okinawa'		140	200	167	507
'Okinawa'		229	129	124	482
	Total	649	547	291	1487
	Average	216	182	97	496
'Nemaguard'		111	0	0	111
'Nemaguard'		124	94	218	436
'Nemaguard'		141	106	59	306
	Total	376	200	277	853
	Average	125	67	92	284
	Total	1474	1092	820	

Experiment II

Above-ground plant symptoms caused by nematode injury were not observed during this experiment making it impossible to distinguish between inoculated and uninoculated plants. But P. brachyurus, at the 100 per pot inoculum level on 'Okinawa' peach, reached a density of 3.5 times the original population. 'Lovell' and 'Nemaguard' peach at the 100 inoculum level supported P. brachyurus populations of 2.7 and 2.4 times, respectively, the original inoculum level (Table 5). An analysis of variance showed no statistical significance between root, top, or total dry weights (Table 6).

Experiment II results substantiate that P. brachyurus is capable of reproducing in 'Okinawa,' 'Nemaguard,' and 'Lovell' peach roots. 'Okinawa' again supported the largest nematode population with 'Lovell' and 'Nemaguard' following in descending numbers.

Small brownish lesions were again observed on roots of plants inoculated with P. brachyurus.

Experiment III

No differences in growth were observed between inoculated and uninoculated plants during this experiment and no symptoms of injury were detected on above-ground plant parts (Fig. 2). At the 1,000 inoculum level, P. brachyurus reproduced 2.4 and 2 times the original population on 'Okinawa' and 'Lovell' peach, respectively (Table 7). 'Nemaguard' was unavailable for use in this experiment.

Fig. 2. Photograph of 'Okinawa' peaches: plant at right inoculated with 1,000 as compared with uninoculated plant.

Table 5. Dry weights of 'Lovell,' 'Okinawa,' and 'Nemaguard' peach seedlings and number of nematodes recovered when uninoculated and inoculated with 100 _Pratylenchus brachyurus_ and grown for 17 weeks in methyl bromide-treated soil.

Peach and Treatment		Dry Weight in Grams			No. of Nematodes
		Root	Top	Plant	
'Lovell' inoculated	1.	1.03	1.13	2.16	268
	2.	1.10	1.16	2.26	232
	3.	1.42	1.49	2.91	290
	4.	.94	1.14	2.08	249
	5.	1.39	1.46	2.85	306
	Total	5.88	6.38	12.26	1345
	Mean	1.18	1.28	2.45	269
'Lovell' uninoculated	1.	1.42	1.47	2.89	0
	2.	1.31	1.38	2.69	0
	3.	1.29	1.32	2.61	0
	4.	1.04	1.07	2.11	0
	5.	1.17	1.22	2.39	0
	Total	6.23	6.46	12.69	
	Mean	1.25	1.29	2.54	

Table 5. (continued)

Peach and Treatment		Dry Weight in Grams			No. of Nematodes
		Root	Top	Plant	
'Okinawa' inoculated	1.	1.41	1.44	2.85	412
	2.	1.22	1.21	2.43	344
	3.	1.10	1.12	2.22	387
	4.	.96	1.01	1.97	322
	5.	1.01	1.07	2.08	260
	Total	5.70	5.85	11.55	1725
	Mean	1.14	1.17	2.31	345
'Okinawa' uninoculated	1.	1.32	1.37	2.69	0
	2.	1.17	1.22	2.39	0
	3.	1.24	1.26	2.50	0
	4.	1.06	1.10	2.16	0
	5.	1.14	1.21	2.35	0
	Total	5.93	6.16	12.09	
	Mean	1.18	1.23	2.42	

Table 5. (continued)

Peach and Treatment		Dry Weight in Grams			No. of Nematodes
		Root	Top	Plant	
'Nemaguard' inoculated	1.	1.21	1.24	2.45	185
	2.	1.32	1.39	2.71	139
	3.	1.04	1.06	2.10	315
	4.	1.47	1.51	2.98	260
	5.	.94	.99	1.93	306
	Total	5.98	6.19	12.17	1205
	Mean	1.20	1.24	2.43	241
'Nemaguard' uninoculated	1.	1.47	1.52	2.99	0
	2.	1.40	1.45	2.85	0
	3.	1.13	1.14	2.27	0
	4.	1.29	1.35	2.64	0
	5.	1.62	1.62	3.24	0
	Total	6.91	7.08	13.99	
	Mean	1.38	1.42	2.80	

Table 6. Analysis of variance

Source of variation	df	Sum of Squares	Mean Square	F
Analysis of variance: 'Lovell' top dry weight in grams (from data of Table 1)				
Treatment	1	.002	.002	.07547
Error	8	.212	.0265	
Total	9	.214		
	P. brachyurus 6.38		uninoculated 6.46	
Analysis of variance: 'Lovell' root dry weight in grams (from data of Table 1)				
Treatment	1	.011	.001	.314
Error	8	.279	.035	
Total	9	.290		
	P. brachyurus 5.88		uninoculated 6.23	

Table 6. (continued)

Source of variation	df	Sum of Squares	Mean Square	F
Analysis of variance: 'Lovell' total dry weight in grams (from data of Table 1)				
Treatment	1	.186	.186	1.90
Error	8	.784	.098	
Total	9	.97		

P. brachyurus	uninoculated
12.26	12.69

Source of variation	df	Sum of Squares	Mean Square	F
Analysis of variance: 'Okinawa' root dry weights in grams (from data of Table 1)				
Treatment	1	.011	.011	.52
Error	8	.169	.021125	
Total	9	.180		

P. brachyurus	uninoculated
5.70	5.93

Table 6. (continued)

Source of variation	df	Sum of Squares	Mean Square	F
Analysis of variance: 'Okinawa' top dry weight in grams (from data of Table 1)				
Treatment	1	.013	.013	.76
Error	8	.137	.017	
Total	9	.15		

P. brachyurus	uninoculated
5.85	6.16

Source of variation	df	Sum of Squares	Mean Square	F
Analysis of variance: 'Okinawa' total dry weight in grams (from data of Table 1)				
Treatment	1	.034	.034	.43
Error	8	.626	.078	
Total	9	.66		

P. brachyurus	uninoculated
11.55	12.09

Table 6. (continued)

Source of variation	df	Sum of Squares	Mean Square	F
Analysis of variance: 'Nemaguard' root dry weight in grams (from data of Table 1)				
Treatment	1	.086	.086	2.34
Error	8	.294	.036	
Total	9	.380		
	P. brachyurus 5.98		uninoculated 6.91	
Analysis of variance: 'Nemaguard' top dry weights in grams (from data of Table 1)				
Treatment	1	.079	.079	1.92
Error	8	.329	.041	
Total	9	.408		
	P. brachyurus 6.19		uninoculated 7.08	

Table 6. (continued)

Source of variation	df	Sum of Squares	Mean Square	F
Analysis of variance: 'Nemaguard' total dry weight in grams (from data of Table 1)				
Treatment	1	.33	.331	4.96
Error	8	.53	.066	
Total	9	1.86		

P. brachyurus	uninoculated
12.17	13.99

Table 7. Dry weights of 'Lovell' and 'Okinawa' peach seedlings and numbers of nematodes recovered when uninoculated and inoculated with *Pratylenchus brachyurus* and grown for 17 weeks in methyl bromide-treated soil

Peach and Treatment		Dry Weight in Grams			No. of Nematodes
		Root	Top	Total	
'Lovell' inoculated	1.	.92	1.09	2.01	2140
	2.	.97	1.21	2.18	1979
	3.	.89	1.10	1.99	2244
	4.	1.01	1.17	2.18	1715
	5.	1.10	1.26	2.36	1827
	Total	4.89	5.83	10.72	9905
	Mean	.978	1.17	2.14	1981
'Lovell' uninoculated	1.	1.20	1.29	2.49	0
	2.	1.37	1.44	2.81	0
	3.	1.09	1.14	2.23	0
	4.	1.32	1.40	2.77	0
	5.	1.04	1.07	2.11	0
	Total	6.07	6.34	12.41	
	Mean	1.21	1.27	2.48	

Table 7. (continued)

Peach and Treatment		Dry Weight in Grams			No. of Nematodes
		Root	Top	Total	
'Okinawa' inoculated	1.	.96	1.02	1.98	2319
	2.	.89	.97	1.86	2614
	3.	.94	1.01	1.95	1974
	4.	.76	.90	1.66	2149
	5.	1.02	1.10	2.12	2730
	Total	4.57	5.00	9.51	11,786
	Mean	.91	1.00	1.91	2357
'Okinawa' uninoculated	1.	1.29	1.34	2.63	0
	2.	1.17	1.26	2.43	0
	3.	1.15	1.24	2.39	0
	4.	1.06	1.12	2.18	0
	5.	1.24	1.30	2.54	0
	Total	5.91	6.26	12.17	
	Mean	1.18	1.25	2.45	

Statistical analysis of data obtained from this experiment and the analysis of variance table show that top, root, and total dry weights of 'Okinawa' plants inoculated with P. brachyurus are statistically different from uninoculated plants at the 1 per cent level of probability (Table 8). This statistical method also shows that root weights of inoculated and uninoculated 'Lovell' plants were statistically different at the 5 per cent level of probability (Table 8). No statistically significant difference existed at either the 1 or 5 per cent levels of probability when top dry weights or total dry weights of 'Lovell' peach were compared.

Obvious disease symptoms such as stunting, chlorosis, and leaf drop being absent on test plants suggests that injury to peach is very gradual and subtle.

Histopathology of Peach Roots Infected With *Pratylenchus brachyurus*

Microscopic examination of sections of infected roots revealed the presence of P. brachyurus within and damage to cortical regions (Fig. 4). Various developmental stages of the parasite were found within root tissues showing various stages of damage. Eggs were noted singly and in groups of three to four inside single cavities. Tissue damage varied from slight effects on several cells to the formation of small cavities due to destruction of many cells (Fig. 5). Small root lesions and ruptures were obvious in root epidermis in some instances, but injury was not extensive.

Fig. 3. Photomicrograph of a transverse section of 'Okinawa' peach root from uninoculated plant.

Fig. 4. A-B. Photomicrograph of a transverse section of 'Nemaguard' peach root infected by Pratylenchus brachyurus. A. Portions of nematode bodies (arrows) are adjacent to cavities in the cortical region.

B. Head portion of a nematode in cortical region. Note the absence of cavity formation.

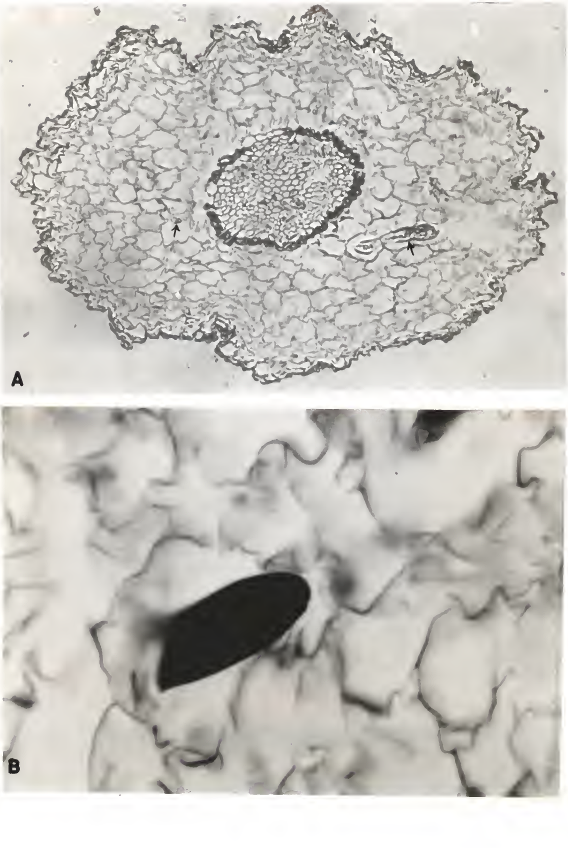

A
B

Fig. 5. A-B. Photomicrograph of a transverse section of 'Okinawa' peach root infected with *Pratylenchus brachyurus*. A. Portion of a nematode (a) is in one of the cortical cavities (b). Note proximity of cavities to epidermis.

B. Head portion of a nematode in cortical region near epidermis.

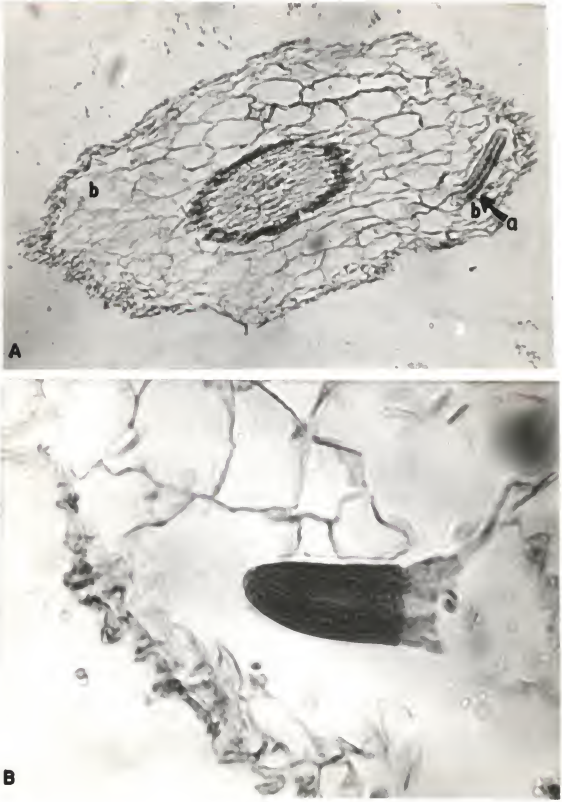

b
b
a
A
B

Table 8. Analysis of variance

Source of variation	df	Sum of Squares	Mean Square	F
Analysis of variance: 'Lovell' top dry weight in grams (from data of Table 4)				
Treatment	1	.0101	.0101	.50
Error	8	1.80901	.202	
Total	9	1.81911		
	P. brachyurus inoculated		uninoculated	
	5.83		6.34	
Analysis of variance: 'Lovell' root dry weight in grams (from data of Table 4)				
Treatment	1	.13924	.13924	8.66**
Error	8	.12865	.01608	
Total	9	.26789		
	P. brachyurus inoculated		uninoculated	
	4.89		6.07	

Table 8. (continued)

Source of variation	df	Sum of Squares	Mean Square	F
Analysis of variance: 'Lovell' total weight in grams (from data of Table 4)				
Treatment	1	.28561	.28561	4.81
Error	8	.47470	.05933	
Total	9	.76031		
	P. brachyurus inoculated		uninoculated	
	10.72		12.41	
Analysis of variance: 'Okinawa' top weight in grams (from data of Table 4)				
Treatment	1	.15876	.15876	24.20***
Error	8	.05248	.00656	
Total	9	.21124		
	P. brachyurus inoculated		uninoculated	
	5.00		6.26	

Table 8. (continued)

Source of variation	df	Sum of Squares	Mean Square	F
Analysis of variance: 'Okinawa' root weight in grams (from data of Table 4)				
Treatment	1	.179560	.179560	25.02***
Error	8	.0574	.007175	
Total	9	.23696		
	P. brachyurus inoculated		uninoculated	
	4.57		5.91	
Analysis of variance: 'Okinawa' total weight in grams (from data of Table 4)				
Treatment	1	.6760	.6760	25.60***
Error	8	.2112	.0264	
Total	9	.8872		
	P. brachyurus inoculated		uninoculated	
	9.57		12.17	

**Significant at 5 per cent level.

***Significant at 1 per cent level.

The nematodes observed inside peach roots were mostly oriented along the transverse root axis with heads away from root tips. Occasionally, the heads were observed at right angles to the transverse axis. The stele was not generally infected; however, in two instances single specimens of *P. brachyurus* were found inside the stele (Fig. 6).

Roots Stained With Safranin-Fast Green

The walls of plant cells not affected by the feeding actions of *P. brachyurus* appeared green as compared to a red color of the cell walls associated with feeding by the nematodes. Cytoplasm of cells affected by nematode feeding was more granular than that of normal cells. Apparent nematode injury extended sometimes two or three cells away from the nematode, which is suggestive of chemical damage by nematode secretions. Safranin typically stains lignified and cutinized cell walls a brilliant red; whereas, fast green should be prominent on cellulose cell walls (14). No evidence of hypertrophy or hyperplasia was observed.

The damage caused by *P. brachyurus* to each of the three peach rootstocks was quite similar. There were no apparent differences in the kind or degree of damage caused by the feeding of the different species of nematodes on the root tissue. Both mechanical and chemical factors appeared involved in total plant damage. Cavities located in the cortical parenchyma resulted from feeding and migration of the nematode. Injury to the vascular system was limited to the cells occupied by the nematode or being fed upon.

The ability of *Pratylenchus brachyurus* to successfully parasitize and reproduce on 'Lovell,' 'Okinawa,' and 'Nemaguard' suggests adequate nutrition furnished by this host to the nematodes. There

Fig. 6. Photomicrograph of a longitudinal section of 'Lovell' peach root infected with Pratylenchus brachyurus. Head of nematode (arrow) is inside the stele.

was no evidence in Pratylenchus brachyurus-infected peach to indicate presence of a "walling off" process (20), a mechanical type of plant resistance that develops when Meloidogyne javanica feeds on 'Okinawa' or 'Nemaguard,' or any other type of plant resistance. "Walling off" causes a breakdown of giant cells which in effect removes the nematode's food source. This type root-knot nematode resistance does not occur in 'Lovell' peach. P. brachyurus reproduced to large numbers on 'Lovell,' 'Okinawa,' and 'Nemaguard,' but caused slight root damage. Further testing of the three rootstocks is needed to determine the effects of longer exposures to large numbers of P. brachyurus.

P. brachyurus normally fed solitary in the cortical region of peach; however, nematodes in groups of two to three occasionally were observed.

As many as six P. brachyurus in solitary sites were observed in a single transverse peach root section, but in no case was "nesting" found in peach roots, even where cortical necrotic cavities occurred.

"Nesting" often occurs in necrotic cavities resulting from the feeding of this species of Pratylenchus.

Study of Pratylenchus brachyurus From Experimental Plants

Measurements and characteristics of P. brachyurus recovered from peach roots in Experiments I, II, and III conformed to those described by Sher and Allen (27), but males differed from those described by Brooks and Perry (1) from citrus in Florida. In more than 100 males examined there was no extension of the phasmid into the bursa (Fig. 7), in agreement with Sher and Allen (27). Brooks and Perry (1) reported

Fig. 7. Photomicrograph of the male tail of Pratylenchus brachyurus. No bursal rib is present.

that the phasmid on males from citrus extended into the bursa. Even when the experimental peach population was reared on citrus, males maintained their bursal characteristic forming no phasmidial rib. Thus two discrete populations of P. brachyurus exist in Florida; however, these differences do not warrant species separation at this time. Males of the peach population apparently do not inseminate females, as no sperm were observed in the uteri of the females.

Study of Pratylenchus penetrans and Pratylenchus coffeae From Experiment I

Morphological and dimensional characteristics of P. penetrans and P. coffeae recovered from experimental plants conformed to the species descriptions by Sher and Allen. Males recovered from experimental plants comprised approximately 25 per cent and 35 per cent of the total populations of P. coffeae and P. penetrans, respectively.

Fungus Associated With Nematode Colony

Populations of Pratylenchus brachyurus were maintained on 'Lovell,' 'Nemaguard,' and 'Okinawa' peach in a greenhouse for biotype identity in 8-inch clay pots containing a soil mixture of approximately one-third peat and two-thirds sandy soil by volume. A periodic examination of soil and peach roots revealed a fungus associated with certain specimens of P. brachyurus. A fungus structure of the constricting ring type often encircled nematodes in the region of the stylet knob. Other nematodes without the constricting ring fungus were constricted in the stylet knob region as evidence of previous association with the fungus.

Three nematodes trapped in a constricting ring with fungus mycelium attached were placed on Petri plates containing 2 per cent potato-dextrose agar. Cultures of the fungus grown on this agar were transferred to 10 x 50 mm glass test tubes containing 2 per cent corn meal agar plus dextrose. Characteristics of the fungus conformed to the description of Arthrobotrys anchonia Drechsler. Type material of this fungus was collected in Ft. Lauderdale, Florida (7).

A. anchonia (Fig. 8-A) was reported (7) to trap and parasitize Eucephalobus sp., but it has never before been reported to trap a plant parasitic nematode (Fig. 8-B).

Stylet Knob Variation in a Colony of Pratylenchus brachyurus

Colonies of P. brachyurus maintained in a greenhouse were examined periodically, particularly to determine nematode vigor, presence of contaminants, or nematode predators and diseases. One collection of P. brachyurus colonized on 'Dixie 18' field corn contained specimens exhibiting an atypical morphological characteristic of the stylet knobs of the species. The typical shape of stylet knobs of P. brachyurus is spheroid to oval (Fig. 9). Abnormal anterior projections of varying lenths (Fig. 10 A-F and a-f) were formed on one to all three stylet knobs of the atypical specimens grown on 'Dixie 18' corn. These stylet knobs appeared in lateral view to have apical projections. In no instance was the projection observed extending anteriorly more than half the stylet length. Margins of the stylet knob extensions were within the marginal bounds of the stylet extruder

Fig. 8. A-B. Photomicrographs of _Arthrobotrys anchonia_ showing constricting rings. A. Constricting rings are attached to hyphal strands.

B. _Pratylenchus brachyurus_ female caught in constricting ring.

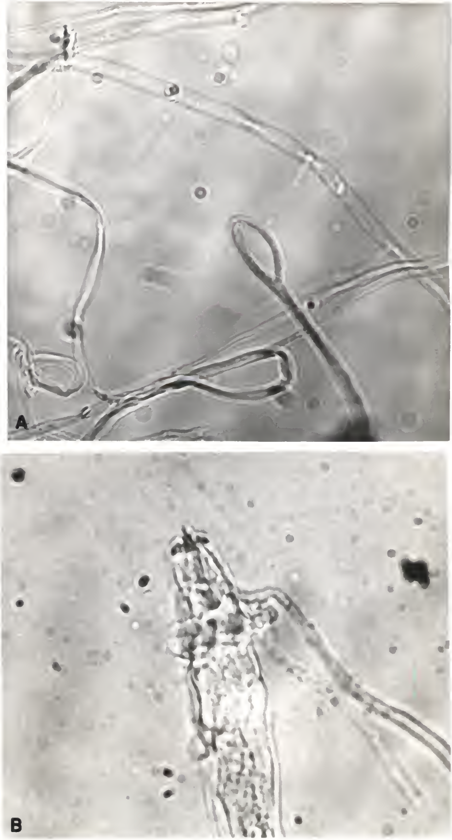
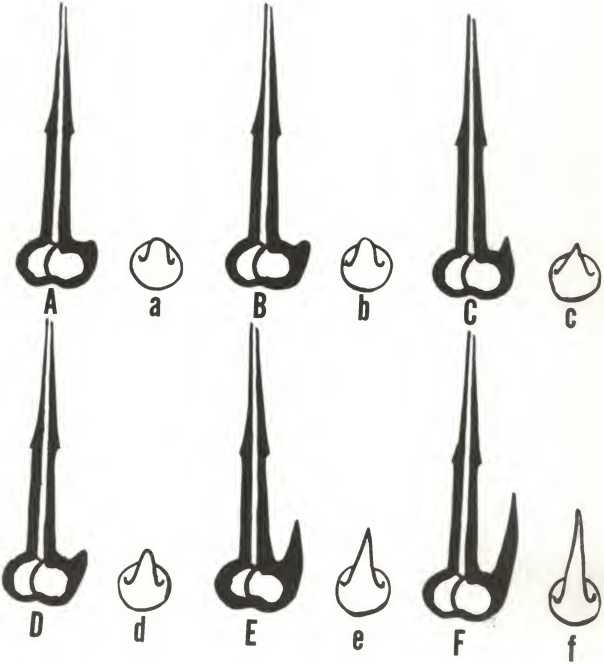

A
B

Fig. 9. Normal Pratylenchus brachyurus stylet showing spheroid knobs.

Fig. 10. A-F, a-f. Lateral views (A-F) of variations in stylet knob abnormalities of Pratylenchus brachyurus correspond to sub-ventral views of malformities (a-f).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muscles. The abnormal stylet occurred on approximately 40 per cent of the females in certain colonies on 'Dixie 18' field corn but not in all colonies. The abnormality was not found on _P. brachyurus_ reared on other hosts, and progeny of females possessing the abnormal stylet characteristic reared on peach or citrus did not have the atypical condition.

CONCLUSIONS

1. Plant parasitic nematodes belonging to the genus *Pratylenchus* have been recovered from roots of declining peach trees.
2. Three species of this nematode genus, *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev and Schuurmans Stekhoven, 1941, *Pratylenchus coffeae* (Zimmerman, 1898) Goodey, 1951, and *Pratylenchus penetrans* (Cobb, 1917) Chitwood and Oteifa, 1952, will parasitize and reproduce on peach, *Prunus persica* Batsch: 'Lovell,' 'Nemaguard,' and 'Okinawa' rootstocks.
3. *Pratylenchus brachyurus* reproduced a greater population than either *P. coffeae* or *P. penetrans* in one inoculation test suggesting greater potential damage to hosts.
4. Damage by *Pratylenchus brachyurus* to peach roots is typified by lesions on epidermal tissues and injury to cortical regions.
5. Levels of inoculum influence severity of *P. brachyurus* damage to 'Okinawa' rootstock. Longer exposures of the host to the nematode could influence severity in plant injury, particularly with perennial crops such as peach in areas with little seasonal climatic variation. It is possible that population thresholds were not reached in 17 weeks.
6. A variable stylet characteristic among *Pratylenchus brachyurus* populations occurred in certain populations.

7. A ring-forming fungus, *Arthrobotys anchonia* Drechsler, is capable of ensnaring *Pratylenchus brachyurus*; however, the efficiency of this fungus to control the nematode is unknown.

8. *Pratylenchus brachyurus* males reared on peach lack a small rib formed by phasmids extending into the bursa and differ from *P. brachyurus* reared on citrus in this respect.

SUMMARY

P. brachyurus occurs in peach nurseries and orchards in Florida, but its possible role in peach decline was heretofore undefined. An initial test was established to ascertain parasitism of *P. brachyurus* to 'Lovell,' 'Okinawa,' and 'Nemaguard' peach rootstocks. *P. brachyurus* was more prolific than *P. penetrans* or *P. coffeae* on these hosts. Two pathogenicity tests of 17 weeks duration were conducted using 100 or 1,000 specimens of *P. brachyurus* per plant, each treatment being replicated five times. 'Okinawa,' 'Nemaguard,' and 'Lovell' peach were used in the first test, but 'Nemaguard' was not available for the second. The parasite reproduced at rates of 2.7, 3.5, and 2.4 times the inoculum level on 'Lovell,' 'Okinawa,' and 'Nemaguard' peach, respectively (low inoculum level test) and 2.4 and 2 times on 'Okinawa' and 'Lovell', respectively (high inoculum level test). Temperatures of 24 ± 2 C and a photoperiod at 2200 ft c for 16 hrs were maintained throughout all tests.

Statistical comparisons of plant dry weights (top, root, and total) disclosed no significant difference between inoculated and uninoculated plants except in the high inoculum level test. Dry weights of top, roots, and total plant of 'Okinawa' peach were statistically significant at the 1 per cent level; with 'Lovell' the dry weight of the roots only was significantly different from the control and that was at the 5 per cent level.

Microscopic examination of sections of each peach rootstock revealed damage to cortical parenchyma by *P. brachyurus*. Tissue damage varied from slight effects on several cells to formation of small cavities due to cell destruction.

Results indicate that even large numbers of *P. brachyurus* may have little effect on early growth of 'Okinawa' and 'Lovell' peach seedlings.

Abnormal stylet knob extensions were discovered on some colonies of *P. brachyurus*.

Arthrobotrys anchonia ensnared *P. brachyurus* specimens in colonies maintained on peach growing in soil containing about one-third peat.

Pratylenchus brachyurus males reared on peach did not have a small rib formed by the phasmids extending into the bursa which is characteristic of *P. brachyurus* males found on citrus in Florida.

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BIOGRAPHICAL SKETCH

Donald Eugene Stokes was born August 25, 1931, at Andalusia, Alabama. After attending public schools in DeFuniak Springs, Florida, he enrolled in the University of Florida, where he received the degree of Bachelor of Science in Agriculture in January, 1956. He was employed by the State Plant Board of Florida in 1956 and served as District Inspector until he entered the Graduate School in 1962. He received a Master of Agriculture degree in April, 1963. He continued graduate work toward the Doctor of Philosophy degree while continuing employment with the Division of Plant Industry, Florida Department of Agriculture and Consumer Services (formerly the State Plant Board) until completion of requirements for the degree in June, 1972.

He is married to the former Alma Grace Pollman, and has five children: Mary Donna, Jack, Nelle, Bertha, and Susan. He is a member of Alpha Zeta, Phi Sigma, the Society of Nematologists, the European Society of Nematologists, the Organization of Tropical American Nematologists, The Florida Nematology Forum, and the Florida State Horticulture Society.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Vernon G. Perry

Vernon G. Perry, Chairman
Professor of Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Grover C. Smart, Jr.

Grover C. Smart, Jr.
Associate Professor of Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

June, 1972

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